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ABSTRACT

This module, one of 25 on vocational education training for careers in environmental health occupations, contains self-instructional materials on performing analyses for waterborne bacteria. Following guidelines for students and instructors and an introduction that explains what the student will learn are three lessons: (1) naming, sterilizing and assembling the component parts of a membrane filter test unit; (2) suctioning a water sample through a membrane filter, using aseptic techniques, and preparing the membrane filter for incubation; and (3) determining the number of total coliform organisms in a water sample. Each lesson contains objectives, recommended methods and locations for practice, performance criteria, equipment and supplies to perform a task, detailed step-by-step instructions for learning a task, and performance exercises. Two performance tests cover preparing water samples for bacteriological analysis and determining the number of micro-organisms in a water sample. (CT)

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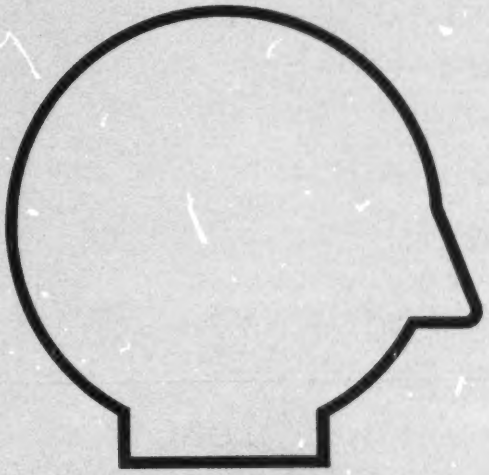

Performing Analyses for Waterborne Bacteria

Module 13

U S DEPARTMENT OF HEALTH, EDUCATION & WELFARE
NATIONAL INSTITUTE OF EDUCATION

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CE 029 495

U.S. DEPARTMENT OF EDUCATION, Office of Vocational and Adult Education

FOREWORD

The Curriculum and Instruction Branch of the Office of Vocational and Adult Education, U.S. Department of Education, identified a need to improve the training opportunities for vocational education students interested in pursuing careers in environmental health. To fulfill that need, Consumer Dynamics, Inc., a Rockville, Maryland, based company, was awarded the contract to develop performance-oriented, competency-based modules in the environmental health sciences.

PERFORMING ANALYSES FOR WATERBORNE BACTERIA is one of the modules in the series, "Vocational Education Training in Environmental Health Sciences." The module content is based on selected materials in the environmental health field. The module is intended to supplement existing course materials.

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USING THESE SELF-INSTRUCTION MATERIALS

This self-instruction learning package or module is designed to allow both students and instructors flexibility of use. Although primarily intended for use in existing training programs, the module can be used by anyone interested in learning new skills or perking up old ones. Therefore, two sets of guidelines are presented--one set addressed to students and the other set addressed to instructors. First, find out how you, the student, should use the materials in this book.

GUIDELINES FOR STUDENTS

Take the Performance Test as a pretest.

When you pick up this book and work through it, your goal will not be a letter grade or a high score on an exam. Instead, you will work to develop skills that you can measure. You will not have to worry about how well someone else is doing. Before you start work on this module, you should, first, find out if you have sufficient skills to start training by reading through the section called PERFORMANCE TEST. If you think you can do all or most of the items in this test, ask your instructor to obtain the necessary equipment and supplies. You should have had a course in high school biology, or have gained the equivalent knowledge through on-the-job training; know how to use laboratory pipets, and have learned how to use a stereo microscope.

Work on parts you need to practice.

If you do everything well, according to the criteria in the Performance Test guidelines, you will not need to spend time working on this module. If after taking the Performance Test you discover there are parts you need to practice, follow the key to each item in FOR FURTHER STUDY.

Work straight through each lesson in the order presented.

Should you decide to completely work through this module, begin with the INTRODUCTION and go straight through each of the three lessons. The lesson begins with the OBJECTIVE of the training. Follow the instruction for each part in the order presented. Practice each step in a lesson until you can do it according to the criteria stated for the step. At the end of a lesson, do the EXERCISES. When there are audiovisuals listed at the end of a lesson, ask your instructor for help in obtaining them.

USING THESE SELF-INSTRUCTION MATERIALS

Take the Performance Test as a posttest.

Finally, after you have mastered all of the exercises in each lesson, ask your instructor to watch you do each item in the Performance Test. The items in the Performance Test are intended for use as a posttest to evaluate the quality of your performance. Turn now to the Performance Test.

GUIDELINES FOR INSTRUCTORS

Approach

The approach of these materials is to provide the student with the opportunity to learn skills for using membrane filter filtration equipment, and for determining the number of indicator organisms in a standard sample volume. The equipment you have available may be somewhat different from that presented in the lessons. If such is the case, you may need to write supplementary instructions to point out the equipment differences. The skills tested on the Performance Test are designed for use with any make and model of instrumentation.

Independent Study

Students can work independently and at their own pace. Depending on the time frame you set for completing each lesson, you may want to start a group off in each lesson with a demonstration and informal presentation.

As a Laboratory Workbook

Alternatively, you may choose to use this module as a laboratory workbook in a structured laboratory session. With this option, you may allow students greater access to your assistance, especially in watching them perform the pre- and posttest portions of the training.

General Instructions

Read through each lesson to anticipate what equipment and supplies you will need to make available for students to use. Also, order any audiovisuals or reading materials you think may present a complementary perspective to the training in this module. Use the items in the Performance Test as the minimum requirements for gauging successful completion of the training.

Specific Instructions

Students are required to use sterile, buffered, distilled water. The following instructions from NTOTC* are provided to aid in the preparation of that water:

1. Prepare stock solution of potassium dihydrogen phosphate (KH_2PO_4) by dissolving 34.0 g of this chemical in 500 ml of distilled water and adjusting its pH to 7.2 with 1 N NaOH. Dilute to 1 liter in a volumetric flask.

2. Prepare stock solution of magnesium sulfate ($MgSO_4.7H_2O$) by dissolving 50 g of this chemical in 500-600 ml of distilled water and, after complete dissolving, bring the final volume to 1 liter in a volumetric flask.

3. Prepare working solution of dilution water by adding 1.25 ml of the potassium dihydrogen phosphate stock solution and 5 ml of the magnesium sulfate stock solution to each liter of distilled water to be used in the preparation of dilution water.

4. Sterilize the buffered water in 1-liter quantities. Pour the water into a 1-liter Erlenmeyer flask and stopper with a cotton plug. Autoclave for 15 minutes at 121° C.

*See References.

INTRODUCTION

BACKGROUND

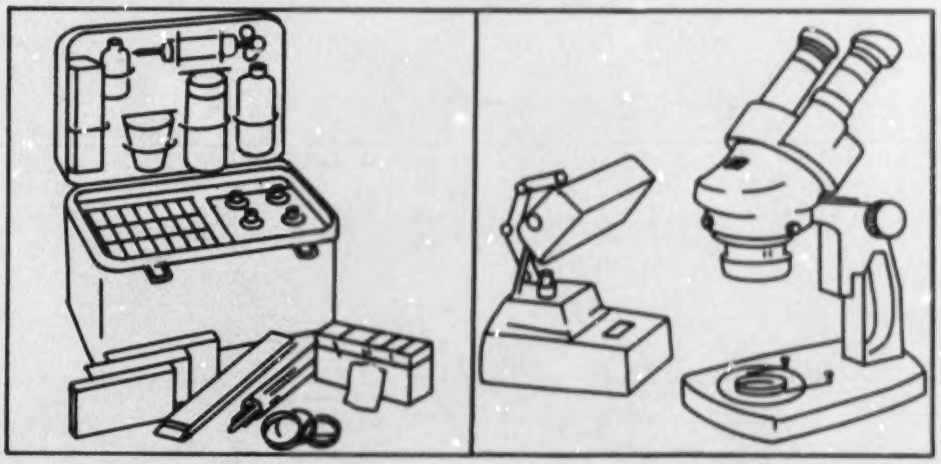
Even before it was known that specific micro-organisms in water cause certain diseases, scientists believed water transferred disease. Microbiologists who study disease causes found that fecal coliform bacteria, normally present in human intestines, are among the micro-organisms that cause cholera, typhoid fever, and dysentery.

The presence of total coliform bacteria, including Klebsiella, Escherichia, Serratia, Envinia, and Enterobacteria are indicators of water quality. But the tests for total coliform bacteria not only indicate the presence of fecal coliform from the intestines of warm-blooded animals but also other coliforms that live in soil. Consequently, total coliform testing is very limited in tracing human disease sources. For this reason, only fecal coliform are tested where nonpotable (nondrinkable) water sources are used for recreational swimming or bathing. Bacteriological testing for total coliform organisms is routinely performed in evaluating the quality of potable water supplies. Therefore, the focus of Lesson Three of this module is on total coliform testing; fecal coliform testing is also included as part of an exercise in this lesson.

There are several methods for performing a microbiological examination for total coliform in drinking water. You will learn the membrane filter technique, which involves suctioning a standard quantity of water through a flexible porous filter. The filter is placed on an absorbent pad containing nutrients on which the bacteria feed during incubation at a constant temperature. The filter can be viewed with the naked eye or with a microscope if there are only a few colonies present; however, if there are many, use of a stereo microscope is recommended. The results of this test indicate the presence of total coliforms (fecal plus nonfecal forms), and their numbers are expressed as bacterial colonies.

WHAT YOU WILL LEARN

When you finish working through the steps and exercises in this module, you will be able to perform an examination of drinking water for the presence of indicator bacteria (coliform).



Using a portable water quality field test kit, you will learn how to perform this function in three lessons:

- o Lesson One

  You will be able to name, sterilize, and assemble the component parts of a membrane filter test unit.

- o Lesson Two

  You will be able to suction a water sample through a membrane filter, using aseptic techniques, and prepare the membrane filter for incubation.

- o Lesson Three

  You will be able to determine the number of total coliform organisms in a water sample.

LESSON ONE

OBJECTIVE

You will be able to name, sterilize, and assemble the component parts of the membrane filter test unit.

WHERE AND HOW TO PRACTICE

In the microbiological testing of water, it is important that it be carried out as aseptically (free from micro-organisms) as possible. You should practice this lesson on a workbench that has been thoroughly cleaned and has room enough to spread out the various components of the filtration unit. The specific testing procedure and equipment you will be using are designed for field use. Under actual conditions you may be required to perform these tests on the tailgate of a station wagon or van. It is important that you learn to minimize any potential sources of contamination.

HOW WELL YOU MUST DO

You must be able to accurately name all the parts of the filtration unit and disassemble, sterilize, and assemble the filtration unit in 20 minutes.

THINGS YOU NEED

You will need the following equipment:

- a portable water laboratory, such as the Millipore Model XX63-001-50 or equivalent,* containing at a minimum:
  - a filtration unit composed of a funnel, membrane filter holding assembly, and a receiving flask
  - a hand-operated or battery-powered pump
  - an alcohol lamp
  - plastic tubing with adaptors
  - sterile, buffered, distilled water, 1,000-ml minimum (see "Guidelines for Instructors")
  - stainless steel forceps

*Use of a particular brand of equipment is not intended to be an endorsement of the product by the U.S. Department of Education.

- sterile, packed petri dishes, absorbent pads, membrane filter

- Endo broth, nutrient media or equivalent, suitable for culturing total coliform

- sterilized stainless steel cup or glass graduated cylinder.

o methanol for sterilizing the filter holder and forceps

o matches.

Instruction 1: Before proceeding with Lesson One, plug in your incubator and let it reach the appropriate incubation temperature for the organism you are culturing.

Instruction 2: Have your instructor demonstrate the proper technique for flaming or sterilizing the forceps.

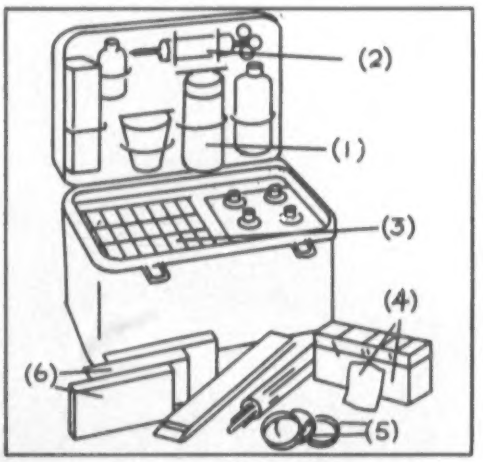
Instruction 3: Now turn to the next page and begin work on Lesson One, "Getting There--Steps."

GETTING THERE--STEPS

STEP 1

Open the portable water lab. Identify the major components of the lab: water filtration unit (1); syringe or hand-operated pump (2) for drawing water through the filtration unit; portable incubator (3) for incubating membrane filters in the field; and a supply of membrane filters (4), petri dishes (5), and nutrient media broth (6). This equipment is suitable for both laboratory and field testing.

KEY POINT 1

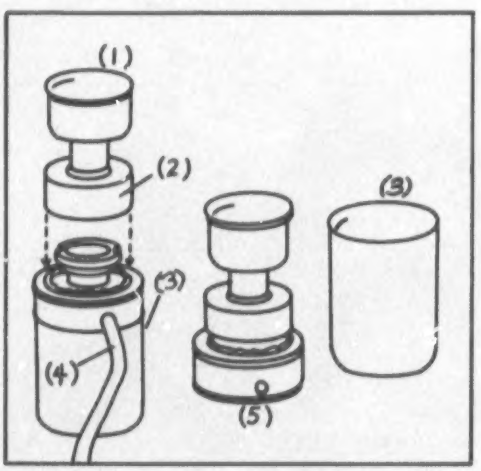


Identify the major components of the portable water lab.

STEP 2

Locate the filtration unit and remove it from the lab kit. The filtration unit is composed of a funnel (1) and a membrane filter holder twist-lock collar (2), enclosed by a stainless steel hydrosol (receiver flask) (3); suction is applied through plastic tubing at the side of the membrane holding assembly (4). Remove the hydrosol by inserting the butt end of the forceps between the hydrosol and the filter holder. Place the hydrosol on the workbench. The funnel and filter holder are shown locked together in (5).

KEY POINT 2

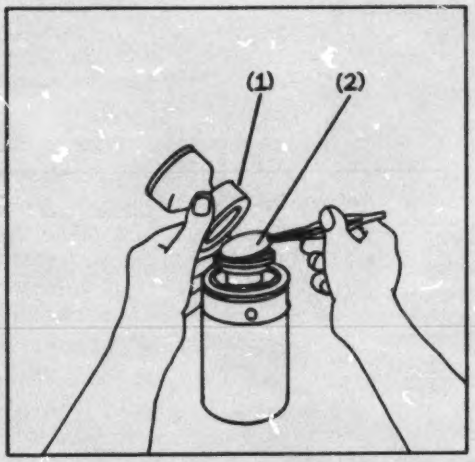


Use the butt end of the forceps to pry open the stainless steel filter holder.

STEP 3

The funnel is secured to the membrane filter holder by a twist-lock collar (1). When tightening or releasing the twist lock, firmly hold the sides of the funnel with one hand and twist the collar counterclockwise to release the funnel from the filter holder. Unscrew the twist lock and remove the funnel. Examine the depression (2) in the holder where a membrane can be placed; then reassemble the funnel to the filter holder base by twisting the collar clockwise.

KEY POINT 3

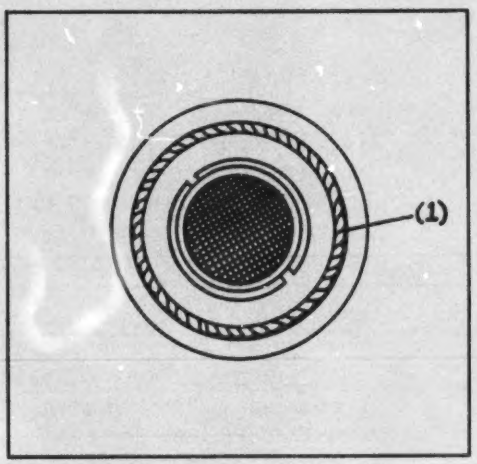


Remove the funnel by unscrewing the twist lock.

STEP 4

Before a water sample can be filtered, the funnel and filter holder must be sterilized. Carefully pour one-half of a capful of methanol on the asbestos ring (1) that surrounds the top of the filter holder base. Do not spill the alcohol down the side or onto the table; flaming may start a fire in your work area.

KEY POINT 4

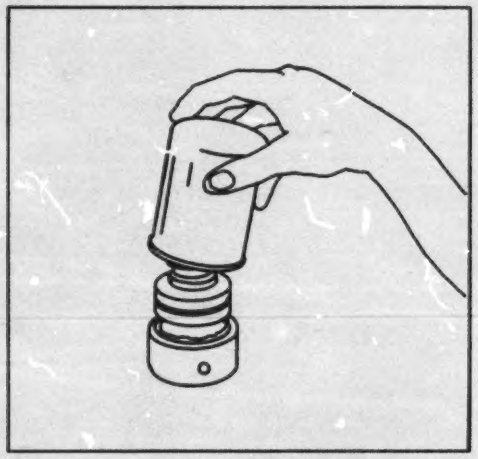


Pour one-half of a capful of methanol on the entire asbestos ring as you rotate the entire unit slowly.

STEP 5

Quickly but carefully light the methanol. Do not lean over the unit when lighting it. Wait 30 seconds and then carefully replace the hydrosol over the funnel and burning asbestos ring. After 2 or 3 minutes, carefully remove the hydrosol and rinse out the funnel thoroughly with sterile, buffered, distilled water to remove any toxic residue. Place the hydrosol under the filter holder to catch the filtered water.

KEY POINT 5

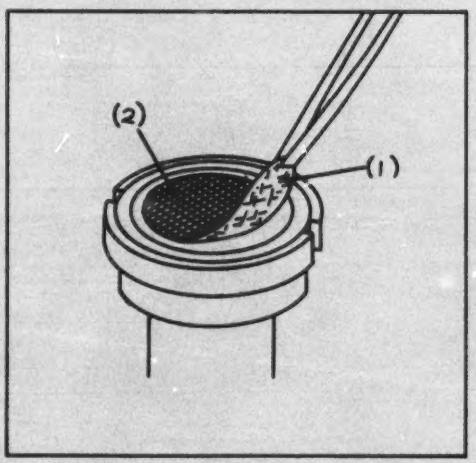


After 30 seconds, replace the receiver flask over the holder flask and wait 2-3 minutes.

STEP 6

Sterilize the forceps by dipping the ends into alcohol and by flaming. Set them on clean dry toweling so the points do not contact the paper. Separate the funnel from the filter holder. Set the funnel on clean dry toweling. Pick up the packet containing the membrane filters and absorbent backup pads. Open the flap and, using the cooled forceps, grasp a flexible filter (white with lines) (1) by the outer 3/16-inch of its circumference. Place the filter, grid side up (2), into the depression in the filter holder.

KEY POINT 6

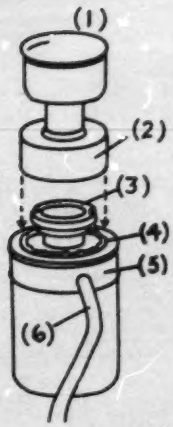


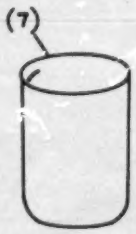
Unlock the sterilized funnel and load the holder with a membrane filter; retain the funnel aseptically.

STEP 7	KEY POINT 7
Carefully replace the funnel and tighten the twist lock. Pick up the hydrosol and attach it to the bottom of the filter holder base. In this position the receiver flask will capture the filtrate (water passing through the membrane filter).	Make sure the twist lock is tightened.

EXERCISES

Instruction 1: Referring to the equipment and/or drawings in the lesson, label the following drawings. You must be able to name each part of a label in the drawing and describe in your own words how a part works or what a label means.





(1) _____

(2) _____

(3) _____

(4) _____

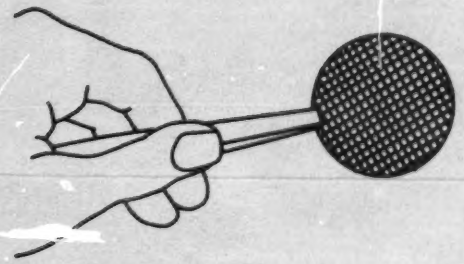
(5) _____

(6) _____

(7) _____

Instruction 2: Practice each step in Lesson One until you can do the following in less than 20 minutes:

- o disassemble, sterilize, and assemble the filtration unit in an aseptic manner
- o flame sterilize a forceps and aseptically grasp a membrane filter by the outer 3/16-inch of its circumference
- o place a membrane filter into the filter holder and assemble the filtration unit.



Instruction 3: When you can perform each of the above correctly without referring to this book or to the manufacturer's operating instructions manual, begin work on Lesson Two.

LESSON TWO

OBJECTIVE

You will be able to suction a water sample through a membrane filter, using aseptic techniques, and prepare the filter for incubation.

WHERE AND HOW TO PRACTICE

This lesson will be carried out in the same location and manner as described in Lesson One. Carefully perform each step in the order given. Any other sequence may prevent an even bacterial dispersion on the membrane filter.

HOW WELL YOU MUST DO

You must be able to suction a water sample through a membrane filter, using aseptic techniques to prevent contamination of the sample by organisms from other samples, or from fecal-contaminated equipment or supplies. You must also be able to disassemble the filter holding assembly, aseptically remove the filter, distribute the media broth evenly on the absorbent pad, and place the filter in the petri dish so that air is not trapped by the filter.

THINGS YOU NEED

In addition to the equipment you used in Lesson One, you will need the following:

- ampules, containing Endo broth media or Endo broth prepared from powdered media
- water, containing fecal organisms, 10-ml minimum
- sterile, graduated pipets and 100-ml capacity cylinder
- clean and dry 4-ounce bottles, 5.

Instructions: Now turn to the next page and begin work on Lesson Two, "Getting There--Steps."

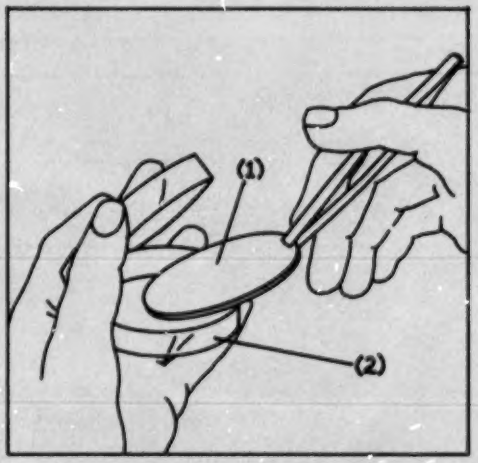
GETTING THERE--STEPS

STEP	KEY POINT
STEP 1 Prepare a positive blank sample, using water from sources containing fecal organisms. Use a sample of raw or unchlorinated sewage water collected in a dry, clean, sterilized 125-ml sample bottle containing sodium thiosulfate. For a label, place a piece of autoclave tape or masking tape on the bottle. Add 1.0 ml of fecal organism containing water to a clean, sterilized bottle and 100 ml of distilled water. Label the bottle: "Positive Blank, 1:100 Dilution."	KEY POINT 1 Prepare a positive blank, sample using water containing fecal organisms.
STEP 2 Prepare a negative blank sample. Obtain a dry, clean, sterilized bottle that contains sodium thiosulfate. For a label, place a piece of autoclave tape or masking tape on the bottle. Label the bottle: "Negative Blank, Distilled Water." Fill the bottle up with distilled water.	KEY POINT 2 Prepare a negative blank sample, using distilled water.

STEP 3

Prepare a petri dish containing an absorbent pad saturated with media broth. Open a pouch containing back-up absorbent pads and filters. Do not handle the pad. Remove a pad (1) with sterilized flamed forceps and place it into the opened petri dish (2) as shown in the Key Point. Break an ampule containing media and distribute it evenly over the surface of the pad. If you prepared media broth in bulk amount, pipet 1.8 ml of sterilized (heated) media onto the pad, using a sterile* pipet.

KEY POINT 3

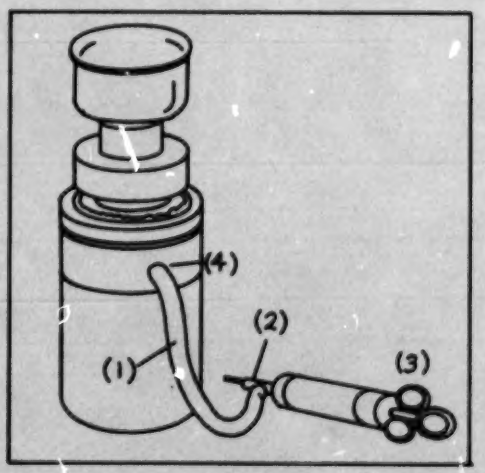


Never touch the absorbent pad.

STEP 4

Attach a piece of suction tubing (1) to the perpendicular side inlet valve (2) of the stainless steel syringe (3). Pump the syringe several times to check for valve blockage. If there is no blockage, insert the plug end of the suction tubing into the side vent hole (4) located in the center of the filter holder base.

KEY POINT 4



Test the syringe before attaching the suction tube to the filter holder.

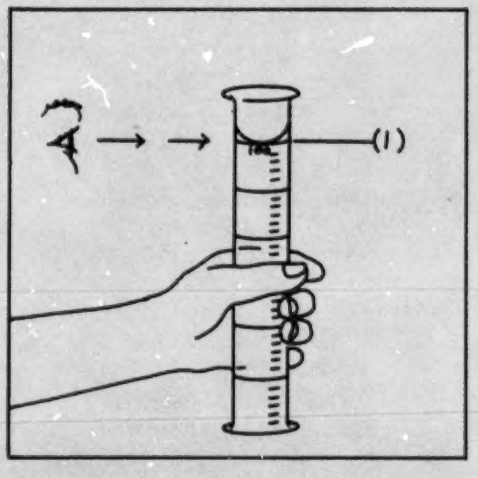
*Free from fecal organisms.

STEP 5	KEY POINT 5
Pour approximately 10 ml of sterile, buffered, distilled water into the funnel. Do not apply suction to the filtering unit. Observe the funnel and holder assembly for leakage. If any leakage occurs, unscrew the unit and inspect the base of the funnel for debris or damage. If the O-ring is damaged, you will not be able to draw suction. Reassemble as in Steps 6 and 7 of Lesson One. If the unit continues to leak, ask your instructor for assistance.	Pour approximately 10 ml of sterile, buffered, distilled water into the funnel.
STEP 6	KEY POINT 6
Using sterilized forceps, remove a membrane filter from its shipping package. Center the filter in the recessed area in the filter holder. Close the filter-pad package and lay it aside in a dry area.	Do not touch the membrane filter at any time.

STEP 7

Locate a clean, sterilized 100-ml graduate cylinder or graduated stainless steel cup provided in the water lab. Uncap the negative blank sample. If you use the graduated cylinder, lift it to eye level and slowly pour 100-ml of the negative blank sample into it until the bottom (1) of the meniscus touches the 100-ml line. Gently pour the 100-ml sample into the funnel. Pour slowly and close to the rim of the funnel to avoid splashing. As a matter of routine practice, rinse the cylinder several times with sterile, buffered, distilled water and pour each rinsing individually into the funnel. Allow a 5-second drainage period before shaking off the last drop.

KEY POINT 7



Observe the meniscus when filling a graduated cylinder.

STEP 8

To generate suction, pump the syringe until all of the sample flows through the membrane and into the receiving flask. The filter will have a semidry look when suction is complete.

KEY POINT 8

Apply suction by pumping with a syringe plunger.

STEP 9

Rinse the funnel three times with sterile, buffered, distilled water. Use about 25 ml for each rinse and flush the walls of the funnel to remove all residual water sample droplets. Then, with the syringe, generate a suction after each rinse and allow all of the water to pass through the membrane before applying the next rinse.

KEY POINT 9

Rinse the funnel with sterile, buffered, distilled water.

STEP 10

After drawing off all the water with the suction, release any negative pressure that may have built up in the filtration unit by gently removing the suction tube adaptor from the filter holder base.

KEY POINT 10

Release negative pressure to prevent damage to the membrane filter.

STEP 11

Holding the funnel with one hand, turn the twist lock and gently separate the funnel from the filter holder. Without touching any of the inside surfaces of the funnel, hold it in one hand while you gently lift the membrane off the filter holder using the flame-sterilized forceps. Once you remove the membrane, temporarily replace the funnel on the filter holder base.

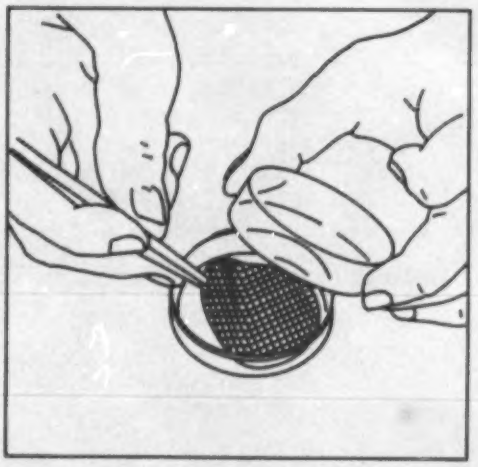
KEY POINT 11

Hold the funnel aseptically.

STEP 12

Remove the cover of the prepared petri dish. With its grid or inked side up, slide the membrane filter across the top of the open petri dish until its far edge just enters the dish and settles on the absorbent pad. Using a slight rolling motion, center the filter on top of the media-saturated absorbent pad. If air pockets occur, pick up the membrane filter by its edge and reroll. If you are still unable to eliminate the air pockets trapped between the filter and the pad, ask your instructor for assistance.

KEY POINT 12



With a rolling motion, slide the membrane filter into the petri dish.

STEP 13

Once the membrane filter is properly positioned, tightly close the petri dish. Remove the tape label from the sample bottle and place it on the petri dish cover. Invert the dish, record the time in your notebook, and then place the inverted dish into a portable incubator with the temperature set at 35 $\pm$0.5° C. This temperature should be maintained for 22-24 hours for culturing total coliform bacteria.

KEY POINT 13

Incubate the inverted petri dish at 35 $\pm$0.5°C for 22-24 hours.

STEP	KEY POINT
14	14
As a routine habit, replace the funnel and rinse the funnel with generous amounts of sterile, buffered, distilled water after suctioning a sample through a membrane filter. Sterilize the forceps after using them.	Rinse the funnel after each use, and flame the forceps after each use.
15	15
Filter the positive blank after the negative blank. Repeat Steps 3 through 13. Water from the following sources shall be filtered in this order: (1) negative blank (2) potable water (3) thawed ice (4) any sample that may yield a high colony count after incubation on media broth (5) positive blank.	Filter first those samples least contaminated.

EXERCISES

Instructions: Check your aseptic technique.

Part A: Prepare Positive Blanks

Obtain five dry, clean, sterilized bottles containing sodium thiosulfate. Replace a piece of autoclave or masking tape on each. Add the following amounts of unchlorinated sewage water:

- Bottle 1 -- 50 ml (1:1 dilution)
- Bottle 2 -- 10.00 ml (1:10 dilution)
- Bottle 3 -- 1.00 ml (1:100 dilution)
- Bottle 4 -- 0.10 ml (1:1,000 dilution)
- Bottle 5 -- 0.01 ml (1:10,000 dilution)

Add enough sterile, buffered distilled water to bottles 1 through 5 to make 100 ml in each bottle. Label each bottle with the dilution ratio.

Part B: Filter Samples

Repeat Steps 3 through 13. Filter bottles in the numbered sequences. In actual practice, do not run less contaminated samples after those that may contain more fecal organisms. See Step 15. Remember to use generous amounts of sterile, buffered distilled water to rinse between samples.

Part C: Incubate Samples

Incubate these samples and use them in Lesson Three. When you count the colonies, you should find that the number of coliform colonies decreases from Bottle 1 through Bottle 5. Without counting the colonies, however, you should see there is a decrease in the total number of organisms present from Bottle 1 through 5. If not, repeat the exercises and pay particular attention to how you handle sterilized equipment and materials, and that you place them in sterile areas when you set them down. You may need to increase the dilution to 1:100,000 by adding 0.01 ml of raw sewage to 1,000 ml of sterile, buffered distilled water. Further dilutions can be made by first diluting the raw sewage water with sterile, buffered distilled water and then taking an aliquot of that and adding it to 100 ml or 1,000 ml of sterile, buffered distilled water.

LESSON THREE

OBJECTIVE

You will be able to determine the number of total coliform organisms in a water sample.

WHERE AND HOW TO PRACTICE

This lesson will be carried out in the same location and manner as described in Lessons One and Two.

HOW WELL YOU MUST DO

You must be able to identify total coliform by their golden metallic sheen with a greenish tint, and generally semispherical shape, and to calculate the density of total coliform colonies present in a 100 ml sample of water.

THINGS YOU NEED

You will need the following equipment:

- wide-field stereo microscope
- fluorescent light source
- petri dishes incubated in Lesson Two.

Instructions: Now turn to the next page and begin work on Lesson Three, "Getting there--Steps."

GETTING THERE--STEPS

STEP 1

After incubating a membrane filter for 22-24 hours at 35 $\pm$0.5° C, carefully remove a petri dish from the incubator. Rough handling or jarring of the plate can cause spattering of droplets and cause difficulty in counting. Gently turn the dish over (right side up). If there are only a few colonies on the filter, you may be able to count them without magnification. Dozens of colonies will require magnification to be read accurately. Take the dish to the microscope area of the workbench to count the colonies.

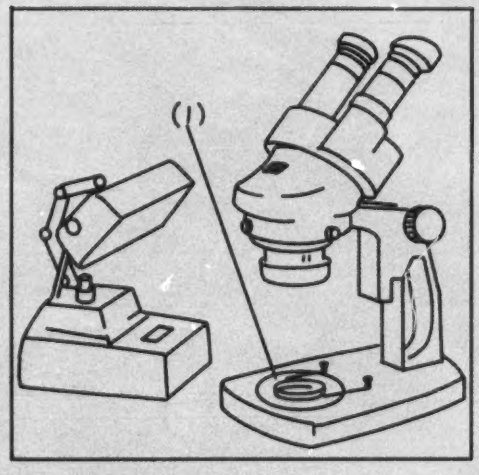
KEY POINT 1

Handle incubated petri dishes carefully.

STEP 2

Remove the cover from the petri dish and place the bottom plate on the stage (1) of a wide-field stereo microscope. Adjust the magnification to 10X. Position and adjust a cool, white fluorescent light source so the light falls as vertically as possible on the membrane filter. (An an angle of 60° to 80° is best.)

KEY POINT 2

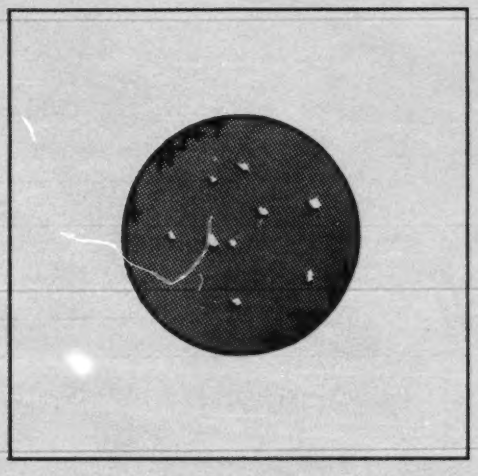


Position the plate bottom on the stage of the stereo microscope under a fluorescent light source.

STEP 3

Look into the stereo microscope and examine the entire surface of the membrane filter for the presence of indicator organisms. The indicator organism colonies are pink to dark red and have a golden metallic sheen with a greenish tint. The metallic sheen may cover an entire colony or only the center. The size of the colonies varies.

KEY POINT 3

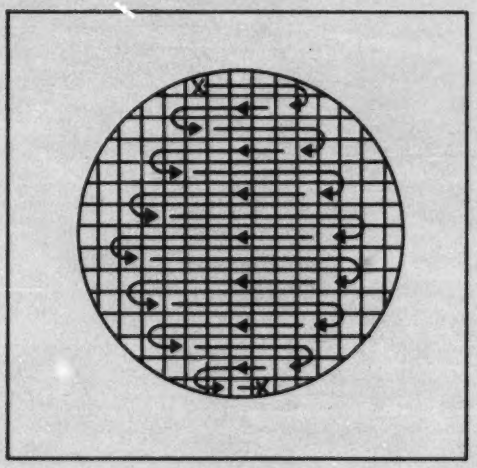


Using a stereo microscope, examine the surface of the membrane filter for the presence of indicator organisms (total coliform).

STEP 4

Microscopically scan the membrane filter with a back and forth movement over the grids and count all colonies having a sheen.

KEY POINT 4

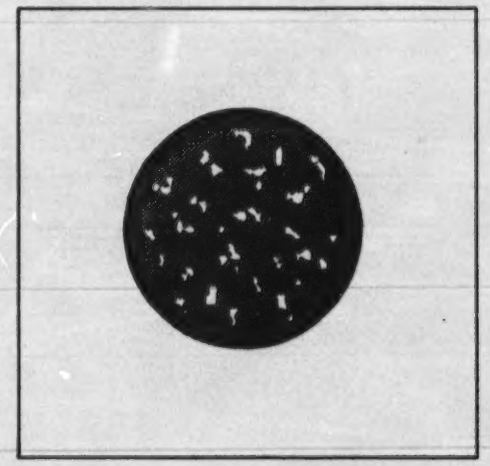


Use the grid system on the surface of the membrane filter to locate colonies along the counting path.

STEP 5

If you observe numerous colonies on the membrane filter, even if they do not have a sheen, you must count these as well; if there is a total colony count (of all type colonies) greater than 200, discard this filter because a count of 200 colonies interferes with the validity of the test. If this occurs on your plate, you will have to dilute your sample the same way you dilute the positive blank to reduce the background colonies. The colonies counted from each dilution series then can be added together to get a total coliform count.

KEY POINT 5



Dilute the sample if the colony count is too high.

STEP 6

Report the colony count per 100 ml of the standard water sample. Refer to the equation in Key Point 6 to get your total colony count. Refer to the water quality standards that apply to your area when determining how many colonies may be present in a sample of potable or recreation water.

KEY POINT 6

$$\frac{\text{Indicator organisms counted} \times \% \text{ dilution} \times 100}{\text{ml filtered sample}} = \text{Indicator organisms/100 ml}$$

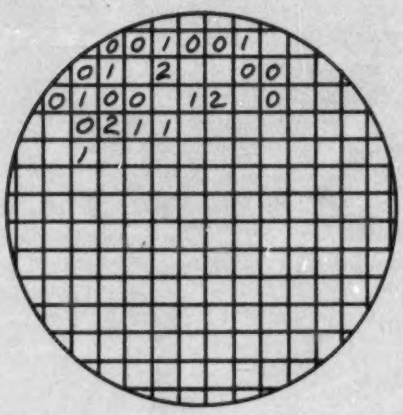
Calculate the density of indicator bacteria present in the sampled water.

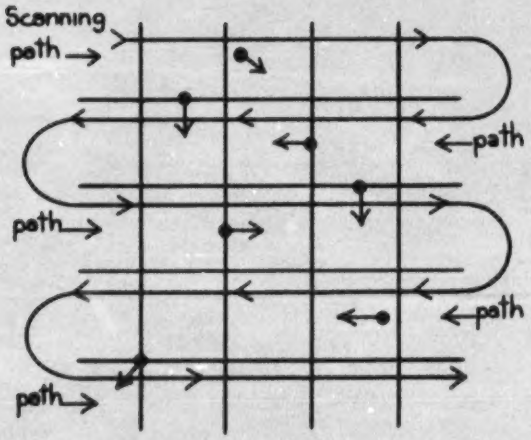
LESSON THREE

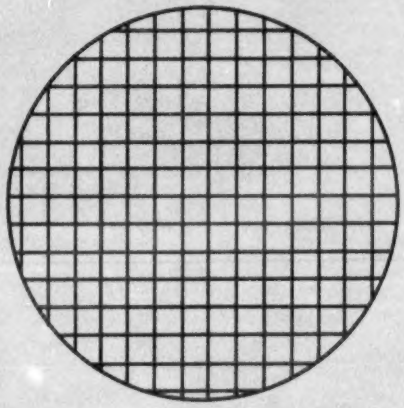
EXERCISES

Instruction 1: Count the total coliform colonies present in the seven petri dishes you incubated in Lesson Two. In calculating the indicator organisms per 100 ml water sample, use the number of milliliters of the contaminated water sample actually used, i.e., 0.01 ml for the 1:10,000 dilution. Using actual counts, verify your findings in Lesson Two/Exercises. If the counts indicate a different result, repeat Lesson Two.

Instruction 2: If there are fewer than 200 colonies, count the total number of bacteria colonies present on an incubated membrane filter and fill in the individual blank squares of the grid below with the number of colonies counted. Use the accompanying diagram to assist in identifying in which square a borderline colony may be located.







Instruction 3: Substitute fecal coliform media broth in the petri dish and incubate your cultures at 44.5 ±0.20° C. This is the test for fecal coliform. When examined under 10-20X magnification, all colonies exhibiting a blue color are fecal coliforms. Calculate the density of fecal coliform colonies present in the water sample using the same formula presented in Key Point 6.

Instruction 4: Count the number of fecal coliform bacteria in your sample (see Instruction 2). Calculate the density of fecal coliform colonies in the water sample.

Instruction 5: Practice each step in Lessons One, Two, and Three at some field location. Also practice using an automobile battery to power your portable incubator and light for your microscope.

PERFORMANCE TEST

Instructions: Check your skill level or progress by working through each of the items in this test. If you can perform each item as required, place a check in the space provided. When all of the items are checked, you are ready to demonstrate your skills to your instructor. You may use the following list if needed. You will be considered trained in a skill after your instructor approves your performance of each of the following items:

PREPARING WATER SAMPLES FOR BACTERIOLOGICAL ANALYSIS

No. 1 _____ Sterlize the funnel and filter assembly, using methanol.

No. 2 _____ Prepare a positive blank sample, using water containing fecal organisms.

No. 3 _____ Prepare a negative blank sample, using sterile, distilled water.

No. 4 _____ Add 1.8 ml Endo broth media (ampule or by pipet from a newly prepared batch) to an absorbent pad in a petri dish so that the media is evenly distributed.

No. 5 _____ Aseptically place a new membrane filter into the filter holder.

No. 6 _____ Filter 100-ml sample and rinse the measuring container and funnel before aseptically removing the membrane filter.

No. 7 _____ Place the semidry membrane filter onto a media saturated pad in a petri dish so that air is not trapped under the filter.

No. 8 _____ Identify the sample, invert it, and incubate it.

No. 9 _____ Prepare the filtration equipment for the next sample.

PERFORMANCE TEST

FOR FURTHER STUDY

If you could not perform one or more of the nine items above, review and practice the following lesson steps:

No. 1
Lesson One, Step 4

No. 2
Lesson Two, Step 1

No. 3
Lesson Two, Step 3

No. 4
Lesson Two, Step 3

No. 5
Lesson Two, Step 6

No. 6
Lesson Two, Steps 7 through 11

No. 7
Lesson Two, Step 12

No. 8
Lesson Two, Step 13

No. 9
Lesson Two, Step 13

DETERMINING THE NUMBER OF MICRO-ORGANISMS IN A WATER SAMPLE

No. 1 _____ Place an incubated sample on the stage of a stereo microscope without damaging the culture.

No. 2 _____ Using a diffuse light source and an appropriate power of magnification, count all coliform colonies, using the scanning technique.

No. 3 _____ Count all colonies up to 200.

No. 4 _____ Calculate the number of indicator organisms per 100 ml of sample.

FOR FURTHER STUDY

If you could not perform one or more of the four items above, review and practice the following lesson steps:

No. 1
Lesson Three, Step 1

No. 2
Lesson Three, Steps 3 and 4

No. 3
Lesson Three, Step 5

No. 4
Lesson Three, Step 6

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